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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

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☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

METHODS FOR TREATMENT OF DIABETES

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☐ Application Data Sheet. See 37 CFR 1.76

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☐ Applicant claims small entity status. See 37 CFR 1.27.

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FILING FEE AMOUNT ($) 80.00

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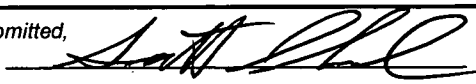
☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE 

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REGISTRATION NO. *(if appropriate)* 51,622

TYPED or PRINTED NAME G. Scott Dorland, Ph.D.

Docket Number: 3154-6270US

TELEPHONE 801-532-1922

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PROVISIONAL PATENT APPLICATION

for

METHODS FOR TREATMENT OF DIABETES

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METHODS FOR TREATMENT OF DIABETES

TECHNICAL FIELD

[0001] The invention relates to medical treatment generally and more particularly to various methods and compositions for the treatment of type II diabetes.

BACKGROUND

[0002] Physostigmines, also called eserines, are known cholinesterase inhibitors. These compounds are also useful in the treatment of glaucoma, Myasthenia Gravis, and Alzheimer's disease, and as antidotes against poisoning with organophosphates.

[0003] The natural isomer of physostigmine has blocking properties, as well as, agonist properties at the neuromuscular acetylcholine receptor (AChR). By contrast, (+)-physostigmine shows only negligible inhibition of cholinesterase (ChE). *See* Brossi et al., FEBS Lett., Vol. 201, pages 190-192 (1986).

[0004] Even though (+)-physostigmine has only negligible ChE inhibitory activity, it is effective as a protective pretreatment drug against multiple lethal doses of sarin, *see* Albuquerque et al, Fundam. Appl. Caltoxicol., Vol. 5, pages 182-203 (1985). The observed beneficial protection appears to be due to direct interactions of the carbamates with the postsynaptic nicotinic AChR. The protective effectiveness of the carbamates against organophosphates appears to be related to the direct ability of the carbamates to decrease the hyperactivation caused by accumulation of the neurotransmitter.

[0005] Diabetes mellitus affects about 17 million Americans and is the 5th leading cause of death by disease in the United States. Direct and indirect medical expenditures attributable to diabetes were estimated at 132 billion US dollars (*see* Hogan *et al.*, (2003) Economic costs of diabetes in the US in 2002, *Diabetes Care*, 26(3):917-932). Direct medical expenditures alone totaled 91.8 billion US dollars and comprised 23.2 billion US dollars for diabetes care, 24.6 billion US dollars for chronic complications attributable to diabetes, and 44.1 billion US dollars for excess prevalence

of general medical conditions (*Id.*). Hogan *et al.* report that in 2002, diabetes more than doubled the cost of health care in the United States. Thus, diabetes imposes a substantial cost burden to society and, in particular, to those individuals with diabetes and their families. Hogan *et al.* stated that "[e]liminating or reducing the health problems caused by diabetes through factors such as better access to preventive care, more widespread diagnosis, more intensive disease management, and the advent of new medical technologies could significantly improve the quality of life for people with diabetes and their families while at the same time potentially reducing national expenditures for health care services and increasing productivity in the U.S. economy" (*Id.* at 917). Therefore, a long felt need exists for improved disease management, particularly, new and improved treatments.

[0006] Diabetes mellitus is also considered a risk factor for the development of vascular dementia, such as Alzheimer's disease (AD). Insulin's role as a neuromodulator in the brain is beginning to emerge, raising a question regarding its significance for AD. Insulin dysregulation may contribute to AD pathology through several mechanisms including decreased cortical glucose utilization particularly in the hippocampus and entorhinal cortex; increased oxidative stress through the formation of advanced glycation end-products; increased Tau phosphorylation and neurofibrillary tangle formation; and increased β-amyloid aggregation through inhibition of insulin-degrading enzyme. Thus, effective treatment of diabetes mellitus may also prevent or slow the onset of vascular dementia.

[0007] It has been reported that erythrocyte membrane protein glycosylation increases by 3.4 fold in diabetes (Dave, Patel, Katyare, (2001) *Indian J. Clinical Biochem.*, 16(1):81-88). However, insulin or sulfonylurea treatment was not reported to reduce the extent of glycosylation (*Id.*). These authors also reported that erythrocyte membrane acetylcholinesterase activity decreased only in the sulfonylurea treated group (*Id.*). In particular, the Vmax of acetylcholinesterase decreased only in the sulfonylurea treated group (*Id.*). While, serum butyrylcholinesterase activity was relatively low in the diabetic and insulin treated diabetic groups, the diabetic state exhibited a decreased Vmax for components I and II of serum butyrylcholinesterase (*Id.*).

Further, these authors report that *in vitro* incubation with insulin differentially affected the Na plus, K plus-ATPase and serum butyrylcholinesterase activities (*Id.*).

[0008] In addition, erythrocyte membrane acetylcholinesterase activity has been reported to be significantly decreased in insulin-dependent diabetes mellitus, with activity negatively correlating with the fasting blood glucose level (*see* Suhail and Rizvi, (1989), Erythrocyte membrane acetylcholinesterase in type 1 (insulin-dependent) diabetes mellitus, *Biochem. J.*, 259:897–899). This report suggested that the number of active enzyme molecules (AChE) in diabetes was reduced.

[0009] Previous studies have also demonstrated that PKC-dependent processes are involved in both ACH-induced Ca2+-signaling and insulin secretion. For example, the neurotransmitter acetylcholine (ACH) increases cytosolic free calcium and is thought to stimulate insulin secretion from pancreatic beta-cells by activating receptors coupled to phosphatidylinositol breakdown, thereby, generating IP3 and diacylglycerol, which activates protein kinase C (PKC).

[0010] Finally, it has been reported that ACh plays a role in the release of hepatic insulin sensitizing substance and the treatment of insulin resistance. This report stated that administration of ACh by a non-liver specific mechanism (intravenous) did not reverse insulin resistance induced by surgical denerveation.

[0011] Thus, the literature provides indefinite and conflicting reports of potential links between acetylcholine, acetylcholinesterase and diabetes, including types I and II.

[0012] Accordingly, there is a need in the art for highly selective agents active *in vivo*, having an acceptable therapeutic window, and minimal side effects, for treatment of diseases, such as diabetes mellitus.

SUMMARY OF THE INVENTION

[0013] The invention relates to a method of treating a subject comprising administering an effective amount of a phenserine compound of the invention or an effective amount of a pharmaceutical composition according to the invention to the subject, *e.g.*, a mammal, such as a human, thought to be in need of such treatment.

[0014] The invention also relates to a method of treating diabetes mellitus and/or the risk of vascular dementia, comprising treating a subject with an effective amount of a phenserine compound, for example, phenserine, ((-)-N-phenylcarbamoyl eseroline), and/or the (+) isomer of phenserine, or a pharmaceutically acceptable salt thereof. A pharmaceutically acceptable salt may be a tartrate, a phosphate, or a fumarate salt.

[0015] The invention also relates to pharmaceutical compositions comprising an effective amount of a phenserine compound or a pharmaceutically acceptable salt thereof, and a method for the treatment of diabetes mellitus and/or the risk of vascular dementia.

[0016] The invention also relates to a method according to the invention comprises administering to the subject an effective amount of a phenserine compound of the invention or a pharmaceutical composition according to the invention, in combination with a hypoglycemic agent selected from the group consisting of sulfonylureas, meglitinides, biguanides, thiazolidinediones, alpha-glucosidase inhibitors, equivalents or mixtures thereof.

[0017] The invention also relates to a method of treating a diabetic condition, for example, a subject's blood glucose levels (hyperglycaemia or hypoglycaemia), carbohydrate intake levels, responsiveness or non-responsiveness to hypoglycemic agents, diabetic neuropathy, diabetic retinopathy, vascular dementia, kidney function, pregnancy, ketone levels, hyperlipidaemia, or coronary artery disease, by administering to the subject an effective amount of a phenserine compound of the invention or a pharmaceutical composition according to the invention.

[0018] The invention further relates to a method of manufacturing a pharmaceutical composition comprising a phenserine compound or a pharmaceutically acceptable salt thereof for the treatment of diabetes mellitus and/or the risk of vascular dementia.

DETAILED DESCRIPTION OF THE INVENTION

[0019] Surprisingly, the phenserine compounds according to the present invention have been found to be useful in the management, treatment and/or prevention

of diabetes. In addition, the phenserine compounds of the invention and derivative salts appear to be less toxic than other carbamate analogues in the art. Further, the phenserine compounds are more brain-targeted versus the rest of the body and are more rapidly cleared from the blood than other AChEIs. Accordingly, the improved method for treating diseases, such as diabetes and/or vascular dementia, using compounds according to the present invention represent a significant advance over the prior art.

[0020] Diabetes mellitus is a disease in which the body does not produce and/or properly use insulin. Improper use of insulin (*e.g.*, insulin resistance) is one of the underlying causes of type II diabetes and may lead to type I diabetes if the pancreatic cells fail due to the insulin secretion demand placed on them. Insulin resistance occurs when the body fails to respond properly to the insulin it already produces. Ninety percent of people with type II diabetes are insulin resistant to some extent. Furthermore, insulin resistance may affect more than 60 million Americans, with one in four of them likely to develop type II diabetes. Additionally, research indicates that insulin resistance is associated with an increased risk for heart disease and stroke.

[0021] Insulin is a hormone that is needed to convert sugar, starches and other food into energy needed for daily life. The most common forms of diabetes are type I (also referred to as insulin depended diabetes or juvenile diabetes) or type II diabetes (also referred to as non-insulin depended diabetes or adult on-set diabetes), although, other classifications exists, for example, diabetes bronze, which typically results from pancreatic damage caused by iron deposition, and gestational diabetes, which typically appears during pregnancy and disappears following birth.

[0022] Currently, there are five distinct classes of hypoglycemic agents available for the treatment of type II diabetes, each class displaying unique pharmacologic properties. These classes are the sulfonylureas, meglitinides, biguanides, thiazolidinediones, and alpha-glucosidase inhibitors. The invention provides another class of agents, (*ie.*, phenserine compounds), useful in the treatment of diabetes, such as type II diabetes. The phenserine compounds may be administered alone or in combination with one or more hypoglycemic agents.

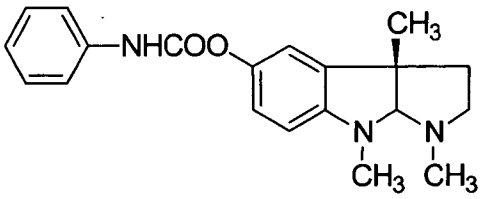
[0023] Acetylcholinesterase inhibitors may be used in the treatment of insulin resistance. However, insulin resistance develops in the liver, therefore, it may be

presumed to be preferable to minimize the diffusion of the acetylcholine esterase agonist into the spinal cord and brian. In particular, administration of ACh targeted to the liver is a logical choice, as opposed to the brain and spinal cord. Thus, the development of insulin resistance in the liver teaches away from the use of the compounds of the present invention, as the phenserine compounds are more brain-targeted versus the rest of the body and are more rapidly cleared from the blood than other AChEIs.

[0024] As used herein, "treatment of diabetes" and the "management of diabetes," are used interchangeably and does not mean a complete cure. It means that the symptoms of the underlying disease are reduced, and/or that one or more of the underlying cellular, physiological, or biochemical causes or mechanisms causing the symptoms are reduced. It is understood that reduced, as used in this context, means relative to the untreated state of the disease, including the molecular state of the disease, not just the physiological state of the disease.

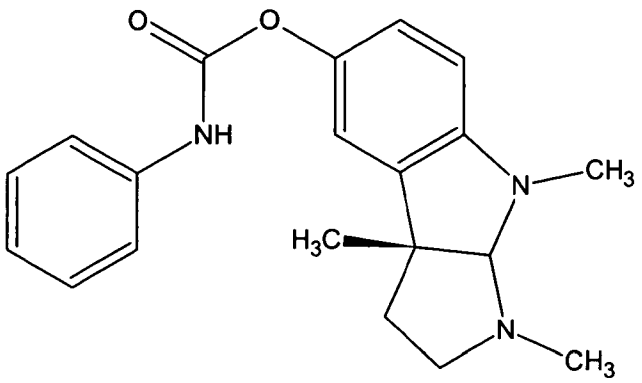
[0025] As used herein, "effective amount" means an amount of an active ingredient administered to the patient, which will be effective to improve or treat the disease condition in the patient.

[0026] Phenserine, (-)-N-phenyl canbamoyleseroline, has the structure:



[0027] Phenserine, ((-)-N-phenylcarbamoyl eseroline), is a carbamate analog of physostigmine (Phy), which is a long-acting inhibitor of cholinesterase. Phenserine was first prepared by Polonovski, (1916), *Bull. Soc. Chim.* 19, 46-59, and technical details were summarized by Beilstein, Handbuch der Organischen Chemie, 4th edn. vol 23. Springer Verlag, Berlin, pp 333 (1954)). It was reported in the literature without any stated practical use.

[0028] In addition, the phenserine compounds of the present invention include the (+) isomer of phenserine, which has the following structure:



II

(3a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl phenylcarbamate

[0029] The phenserine compounds of the invention may be synthesized using processes known in the art. For example, U.S. Patents 6,495,700, 5,409,948, 5,171,750, 5,378,723, and 5,998,460, and WO 03/082270 A1, all of which are hereby incorporated by reference in their entirety, describe the preparation of the phenserine compounds of the invention and enzyme inhibition assays that may be used to test compounds of the invention. The phenserine compounds of the invention are carbamates having specificity for the inhibition of acetylcholinesterase and/or inhibition of β-AAP synthesis, including, but not limited to, (-)-N-phenylcarbamoyl eseroline (which may also be referred to as (3aR)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate); (3aS)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate; (-)-2'-methylphenylcarbamoyleseroline, (-)-2'-4'-dimethylphenylcarbamoyleseroline, (-)-4'-methylphenylarbamoyleseroline, (-)-2'-ethylphenylcarbamoyleseroline, (-)-phenylcarbamoyleserolne, (-)-(-)-2',4',6'-trimethylphenylcarbamoyleseroline, (-)-2'-chlorophenylcarbamoyleseroline, (-)-2',6'-dichlorophenylcarbamoylseroline, (-)-physovenol; (-)-5-O-(2'-methylphenylcarbamoyl)physovenol; (-)-3, 3a, 8, 8a-tetrahydro-3a, 8-dimethyl-2H-thieno-[2,3-b]indole-5-ol butyl carbamate; (-)-3, 3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol heptylcarbamate; (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol phenylcarbamate; (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl;-2H-thieno[2,3-b]indole-5-ol 2'-methylphenylcarbamate; (-)-3,3a,8,8a-tetrahydro-3a,8-dimethy-2H-thieno[2,3-b]indole-5-ol 2'-isopropylphenylcarbamate; (-)-thiaphysovenine,

(-)-Phenyl-thiaphysovenine; (-)-2',4'-dimethylphenyl-thiaphysovenine and pharmaceutically acceptable salts thereof. In addition, physostigmine, physovenine, donepezil, galantamine, rivastigme and tacrine are within the scope of the invention. Salts and free base of the phenserine compounds of the invention are within the scope of the present invention. Salts of phenserine include, but are not limited to, tartrate, phosphate, and fumarate salts.

[0030] Potential cholinesterase agents can be evaluated for potency in vitro by testing the agents against electric eel and human red blood cell acetylcholinesterase (AChE) and human plasma butyrylcholinesterase, (BChE) (*see also*, U.S. Patents 6,495,700; 5,409,948; 5,171,750; 5,378,723; and 5,998,460).

[0031] In an exemplary embodiment, donepezil, galantamine, rivastigme and/or tacrine are used for the treatment of diabetes. These compounds may be used to prevent or reduce insulin resistance and/or to treat dementia associated with βA protein and neurofibrillary tangles. In addition, other AChE inhibitors are known in the art and may also be used according to the methods of the present invention.

[0032] The effect of the cholinesterase agents of the invention may be tested for their potency in the reduction of insulin resistance using methods known in the art, for example, as described in U.S. Patent 5,561,165, RIST, ITT and the HIEC tests (*see*, Reid *et al.*, (2002) Comparison of the rapid insulin sensitivity test (RIST), the insulin tolerance test (ITT), and the hyperinsulinemic euglycemic clamp (HIEC) to measure insulin action in rats, *Can. J. Physiol. Pharmacol.* 80:811-818). Subjects thought to have insulin resistance may be tested using methods known in the art, for example, by the glucose tolerance test or three hour glucose tolerance test.

[0033] The phenserine compounds of the invention are also useful in the treatment of vascular dementia. In an exemplary embodiment, one or more phenserine compounds are used to treat the presence and/or accumulation of the βA protein associated with vascular dementia and/or neurofibrillary tangles.

[0034] Type II diabetes may be a risk factor for dementia, but the associated pathological mechanisms remains unclear. However, diabetes is increasingly associated with total dementia, Alzheimer's disease, and vascular dementia. Individuals with both type II diabetes and the APOE epsilon4 allele have nearly a doubled risk for

AD compared with those with neither risk factor. Subjects with type II diabetes and the epsilon4 allele have a higher number of hippocampal neuritic plaques and neurofibrillary tangles in the cortex and hippocampus, and they have a higher risk of cerebral amyloid angiopathy. Thus, the association between diabetes and AD is particularly strong among carriers of the APOE epsilon4 allele (Peila *et al.*, (2002) Type 2 diabetes, APOE gene, and the risk for dementia and related pathologies: The Honolulu-Asia Aging Study, *Diabetes* 51(4):1256-62). The present invention provides methods of treating diabetes, for example, insulin resistance and/or neurological conditions associated with diabetes.

[0035] Compositions within the scope of the invention include compositions wherein the active ingredient is contained in an effective amount to achieve its intended purpose. Effective concentrations may range from 0.001 wt. % to 1.0 wt, %. The compounds can be administered in any pharmaceutically acceptable amount, for example, in amounts ranging from 0.001 gram to about 1 gram per kilogram of body weight. Based on the information which is presented herein, the determination of effective amounts is well within the skill of the ordinary practitioner in the art. In addition, the ordinary practitioner may formulate the dosage regimen as appropriate for the diabetic condition being treated. For example, the compositions of the invention may be administered orally prior to carbohydrate intake, at times of hypoglycemia or hyperglycemia. Where a compound of the invention is administered prior to carbohydrate intake, the compound may be administered about 3 times a day.

[0036] The compounds are generally used in pharmaceutical compositions (wt %) containing the active ingredient with a carrier, vehicle, diluent and/or excipient in the composition in an amount of about 0.1 to 99 wt % and preferably about 25-85 wt %. Pharmaceutical compositions may be formulated using carriers, diluents and/or excipients known in the art, for example, *see* Goodman and Gilman's: The Pharmacological Basis of Therapeutics (10^{th} ed. 2001). The compounds may be administered in any desired form, including parenterally, orally, injection, transdermally or by suppository using known methods.

[0037] Either fluid or solid unit dosage forms can be readily prepared for oral administration. For example, the active compounds can be admixed with conventional ingredients such as dicalcium phosphate, magnesium aluminum silicate,

magnesium stearate, calcium sulfate, starch, talc, lactose, acacia, methyl cellulose and functionally similar materials as pharmaceutical excipients or carriers. A sustained release formulation may optionally be used where appropriate or desirable. Capsules may be formulated by mixing the compound with a pharmaceutical diluent which is inert and inserting this mixture into a hard gelatin capsule having the appropriate size. If soft capsules are desired, a slurry of the compound with an acceptable vegetable, light petroleum or other inert oil can be encapsulated by forming into a gelatin capsule.

[0038] Suspensions, syrups and elixirs may be used for oral administration of fluid unit dosage forms. A fluid preparation including oil may be used for oil soluble forms. A vegetable oil such as corn oil, peanut oil or sunflower oil, for example, together with flavoring agents, sweeteners and any preservatives produces an acceptable fluid preparation. A surfactant may be added to water to form a syrup for fluid unit dosages. Hydro-alcoholic pharmaceutical preparations may be used having an acceptable sweetener (such as sugar, saccharin, or a biological sweetener, preferably a low carbohydrate sweetener, such as manitol or sorbitol) and a flavoring agent in the form of an elixir.

[0039] Pharmaceutical compositions for parenteral and suppository administration can also be obtained using techniques standard in the art. In an exemplary embodiment, the compounds of the invention are administered as pharmaceutical agents suitable for oral administration. In another exemplary embodiment, the compounds of the invention may be administered by injection in an appropriate vehicle such as sesame oil.

[0040] The pharmaceutical carriers acceptable for the purposes of this invention include all art recognized carriers that do not adversely affect the drug, the host, or the material comprising the drug delivery device. Suitable pharmaceutical carriers include sterile water, saline, dextrose, dextrose in water or saline condensation products of castor oil and ethylene oxide combining about 30 to 35 moles of ethylene oxide per mole of castor oil, liquid acid, lower alkanols, oils such as corn oil, peanut oil, sesame oil and the like, with emulsifiers such as mono- or di-glyceride of a fatty acid; or a phosphatide, *e.g.*, lecithin, and the like; glycols, polyalkylene glycols, aqueous media in the presence of a suspending agent, for example, sodium carboxymethyl cellulose,

sodium alginate, poly(vinylpyrrolidone), and the like, alone, or with suitable dispensing agents such as lecithin, polyoxyethylene stearate, and the like. The carrier may also contain adjuvants such as preserving agents, stabilizing agents, wetting agents, emulsifying agents and the like together with penetration enhancer and the compounds of this invention.

[0041] The effective dose for mammals may vary due to such factors as age, weight, activity level or condition of the subject being treated. Typically, an effective dosage of a compound according to the present invention is about 1 to 800 milligrams when administered by either oral or rectal dose from 1 to 3 times daily. This is about 0.002 to about 50 milligrams per kilogram of the subject's weight administered per day. Preferably about 10 to about 300 milligrams are administered orally or rectally 1 to 3 times a day for an adult human. The required dose is usually considerably less when administered parenterally. Preferably about 0.01 to about 150 milligrams may be administered intramuscularly, one to three times a day for an adult human.

[0042] In an exemplary embodiment, the method according to the invention comprises administering an effective amount of a phenserine compound of the invention or an effective amount of a pharmaceutical composition according to the invention to a subject, such as a mammal, thought to be in need of such treatment. For example, a subject which may benefit from the present invention is a subject suffering from insulin resistance, diabetes and/or vascular dementia. In another exemplary embodiment, the method according to the invention comprises administering to a subject an effective amount of a phenserine compound of the invention or pharmaceutical composition according to the invention, in combination with a hypoglycemic agent selected from the group consisting of sulfonylureas, meglitinides, biguanides, thiazolidinediones, alpha-glucosidase inhibitors or mixtures thereof. In yet another exemplary embodiment, the invention provides a method of preparing a pharmaceutical useful in the treatment of diabetes and/or vascular dementia.

[0043] In an exemplary embodiment, a phenserine compound is administered in combination with an increase in insulin levels. For example, the phenserine may be administered in combination with a bolus of insulin, either an insulin

injection or the action of an agent which stimulates the release of insulin. In another exemplary embodiment, the phenserine compound is administered prior to each meal.

[0044] As will be recognized by a person of ordinary skill in the art, treatment of diabetes, such as type II diabetes, is affected by numerous conditions. For example, the subjects blood glucose levels (hyperglycaemia or hypoglycaemia), carbohydrate intake levels, response to hypoglycemic agents, diabetic neuropathy, diabetic retinopathy, vascular dementia, kidney function, pregnancy, ketone levels, hyperlipidaemia, and coronary artery disease.

[0045] Mice useful in the study of type I diabetes may be obtained, for example, from The Jackson Laboratory Type 1 Diabetes Repository (T1DR) (stocks are available online at jax.org/t1dr/holdings.html). Protocols for the study of diabetes using mouse models are known in the art, for example, as described in Leiter, E.H. Current Protocols in Immunology §§ 15.9.1-15.9.23 (John Wiley & Sons, Inc. eds. 1997). A mouse model for type II diabetes has been described by Fernandez *et al.*, (2001) Functional inactivation of the IGF-I and insulin receptors in skeletal muscle causes type 2 diabetes, *Genes Dev.* 15(15):1926-34.

[0046] Without wishing to be bound by any theory, the phenserine compounds of the invention are believed to inhibit acetylcholinesterase activity, increasing acetylcholine levels, thereby effecting utilization of insulin by a subject. In addition, the compounds of the invention reduce neurofibrillary tangle formation and decrease β-amyloid aggregation, thereby reducing the risk of developing vascular dementia, which is associated with diabetes.

[0047] For example, the presence of insulin in the blood elicits a hepatic parasympathetic reflex, stimulating release of ACh in the liver. The release of ACh releases nitric oxide, which acts to control the sensitivity of skeletal muscle to insulin through the action of a liver released hormone, the hepatic insulin sensitizing substance (HISS). HISS selectively stimulates glucose uptake and storage as glycogen in tissues including skeletal muscle. In the absence of HISS, muscle cells are resistant to insulin and insulin driven storage of glucose by skeletal muscle is reduced.

[0048] HISS release in response to insulin is affected by the fasting state of the subject. Specifically, in the fasting state HISS release is minimal and insulin

produces a minimal metabolic effect. Following a meal, the parasympathetic reflex mechanism is amplified, allowing release of HISS and more efficient utilization of insulin for the storage of glucose in skeletal muscle.

[0049] Decreased release of HISS may result in severe insulin resistance, which may be referred to as HISS-dependent insulin resistance ("HDIR"). In the absence of HISS, the pancreas is required to secrete substantially larger amounts of insulin to compensate for the resistance. Persists insulin resistance is a leading cause of type II diabetes (non-insulin dependent diabetes mellitus) and my lead to a complete exhaustion of the pancreas, thus requiring the patient to resort to insulin injections.

[0050] The phenserine compounds of the invention provide the ability to reduce insulin resistance, thereby providing a treatment for diabetes. Further, the compounds of the invention may be used to prevent cognitive disorders frequently associated with diabetes or reduce the risk of vascular dementia.

Example I

Experimental Protocol for Determining Effect on Insulin Resistance:

[0051] Test animals are anesthetized and an arterial-venous shunt is introduced into the animal according to procedures known in the art. The arterial-venous shunt allows for blood sampling and infusion of test compounds.

[0052] Baseline blood glucose levels are established following surgery. Insulin is then introduced into the animal and glucose infused so as to maintain a steady glucose level throughout the period of insulin activity. By measurement of the glucose infusion rate throughout the experiment, the effect of the insulin is measured.

[0053] The compounds are tested by introducing the test compound at the appropriate time relative to the insulin administration and measuring the effect on glucose infusion. The compounds of the invention increase the rate of glucose infusion relative to control animals.

[0054] For example, phenserine administered approximately 30 minutes prior to the administration of insulin is found to increase the rate of glucose infusion. Thus, phenserine reduces insulin resistance and increases the effectiveness of the administered insulin.

Example II

[0055] Blood glucose levels are established in fasting patients. A predetermined dose of insulin is then administered to the patients followed by feeding the patients meal having a set carbohydrate content. Blood glucose levels are monitored before, during and for at least 4 hours following administration of the insulin.

[0056] The patients are subsequently fasted and the experiment repeated with administration of the test compound prior to administration of the insulin. Comparison of the blood glucose levels for the patients with and without the test compound is performed to determine the effect of the test compound on insulin utilization by the patient.

[0057] Care is taken to avoid hypoglycemic episodes and occurrence of a hypoglycemic episode, in a patient which would not normally experience such an episode, may be taken as evidence of the compounds effect on insulin resistance.

Example III

[0058] β-APP synthesis may be measured *in vitro* or *in vivo* by methods known in the art. For example, by an ELISA assay or a Western. The test compound may be administered to a subject for *in vivo* testing and β-APP levels assayed at various time points.

[0059] Alternatively, cells may be cultured in the presence of a pulse of a labeled amino acid, the label washed off, and the test compound applied to the cells. Label incorporated into the β-APP protein is then quatitated to determine the effect of the compound on the synthesis of β-APP.

[0060] The effect of the test compound on protein synthesis or protein stability may also be determined by other methods known in the art.

[0061] All references, including publications, patents, and patent applications, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0062] While this invention has been described in certain embodiments, the present invention can be further modified within the spirit and scope of this disclosure. This application is therefore intended to cover any variations, uses, or adaptations of the invention using its general principles. Further, this application is intended to cover such departures from the present disclosure as come within known or customary practice in the art to which this invention pertains and which fall within the limits of the appended claims.

CLAIMS

What is claimed is:

1. A method for treating a subject having diabetes, said method comprising:

   administering to a subject at least one phenserine compound and salts thereof, thereby treating diabetes.

2. The method according to claim 1, wherein the phenserine compound is selected from the group consisting of (-)-N-phenylcarbamoyl eseroline, (3aS)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate; (-)-2'-methylphenylcarbamoyleseroline, (-)-2'-4'-dimethylphenylcarbamoyleseroline, (-)-4'-methylphenylarbamoyleseroline, (-)-2'-ethylphenylcarbamoyleseroline, (-)-phenylcarbamoyleserolne, (-)-(-)-2',4',6'-trimethylphenylcarbamoyleseroline, (-)-2'-chlorophenylcarbamoyleseroline, (-)-2',6'-dichlorophenylcarbamoylseroline, (-)-physovenol, (-)-5-O-(2'-methylphenylcarbamoyl)physovenol, (-)-3, 3a, 8, 8a-tetrahydro-3a, 8-dimethyl-2H-thieno-[2,3-b]indole-5-ol butyl carbamate, (-)-3, 3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol heptylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol phenylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl,-2H-thieno[2,3-b]indole -5-ol 2'-methylphenylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8-dimethy-2H -thieno[2,3-b]indole-5-ol 2'-isopropylphenylcarbamate, (-)-thiaphysovenine, (-)-Phenyl-thiaphysovenine, and (-)-2',4'-dimethylphenyl-thiaphysovenine.

3. The method according to claim 2, wherein the phenserine compound comprises (-)-N-phenylcarbamoyl eseroline or a salt thereof.

4. The method according to claim 1, further comprising administering said phenserine compound one to three times daily.

5. The method according to claim 1, further comprising administering said phenserine compound prior to a meal.

6. The method according to claim 1, further comprising coadministering a hypoglycemic agent selected from the group consisting of a sulfonylurea, a meglitinide, a biguanide, a thiazolidinedione, an alpha-glucosidase inhibitor and mixtures thereof.

7. The method according to claim 1, wherein treating diabetes comprises treating at least one condition selected from the group consisting of hyperglycaemia, hypoglycaemia, carbohydrate intake, response to hypoglycemic agents, diabetic neuropathy, diabetic retinopathy, kidney function, vascular dementia, pregnancy, ketone levels, hyperlipidaemia, and coronary artery disease.

8. A method for treating a subject having diabetes, said method comprising:

administering to a subject a pharmaceutically acceptable composition comprising at least one active ingredient selected from the group consisting of (-)-N-phenylcarbamoyl eseroline, (3aS)-1,3a,8-trimethyl-1,2,3,3a,8,8a -hexahydropyrrolo[2,3-b]indol-5-yl phenylcarbamate; (-)-2' -methylphenylcarbamoyleseroline, (-)-2'-4'-dimethylphenylcarbamoyleseroline, (-)-4'-methylphenylarbamoyleseroline, (-)-2'-ethylphenylcarbamoyleseroline, (-)-phenylcarbamoyleserolne, (-)-(-)-2',4',6' -trimethylphenylcarbamoyleseroline, (-)-2'-chlorophenylcarbamoyleseroline, (-)-2',6'-dichlorophenylcarbamoylseroline, (-)-physovenol, (-)-5-O-(2' -methylphenylcarbamoyl)physovenol, (-)-3, 3a, 8, 8a-tetrahydro-3a, 8-dimethyl-2H-thieno-[2,3-b]indole-5-ol butyl carbamate, (-)-3, 3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol heptylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl-2H-thieno[2,3-b]indole-5-ol phenylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8-dimethyl,-2H-thieno[2,3 -b]indole-5-ol 2'-methylphenylcarbamate, (-)-3,3a,8,8a-tetrahydro-3a,8 -dimethy-2H-thieno[2,3-b]indole-5-ol 2'-isopropylphenylcarbamate,

(-)-thiaphysovenine, (-)-Phenyl-thiaphysovenine, (-)-2',4'-dimethylphenyl -thiaphysovenine and pharmaceutically acceptable salts thereof, and a pharmaceutically acceptable diluent, carrier or excipient, thereby treating diabetes.

9. The method according to claim 8, wherein the active ingredient comprises (-)-N-phenylcarbamoyl eseroline or a pharmaceutically acceptable salt thereof.

10. The method according to claim 8, further comprising administering said pharmaceutically acceptable composition one to three times daily.

11. The method according to claim 8, further comprising administering said pharmaceutically acceptable composition prior to a meal.

12. The method according to claim 8, further comprising coadministering a hypoglycemic agent selected from the group consisting of a sulfonylurea, a meglitinide, a biguanide, a thiazolidinedione, an alpha-glucosidase inhibitor and mixtures thereof.

13. The method according to claim 8, wherein treating diabetes comprises treating at least one condition selected from the group consisting of hyperglycaemia, hypoglycaemia, carbohydrate intake, response to hypoglycemic agents, diabetic neuropathy, diabetic retinopathy, kidney function, vascular dementia, pregnancy, ketone levels, hyperlipidaemia, and coronary artery disease.

14. A pharmaceutical composition comprising an acetylecholinesterase antagonist useful in reducing insulin resistance in a mammalian subject suffering therefrom.

15. The pharmaceutical composition of claim 14, further comprising a pharmaceutically acceptable liver-targeting substance.

16. A method of using the composition of claim 14 for the treatment of insulin resistance, said method comprising administering an effective amount of an acetylecholinesterase antagonist to a subject in need thereof.

ABSTRACT

Phenserine compounds and pharmaceutically acceptable salts thereof have been found to be useful in the management or treatment of diabetes and/or vascular dementia. The phenserine compounds of the invention are carbamates that inhibit the activity of acetylcholinesterase.